TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED / ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371	ATTORNEY'S DOCKET NUMBER P67710US0
	US APPLICATION NO (If known, see 37 CFR 1.5) 10/088681
INTERNATIONAL APPLICATION NO PCT/EP00/09241	INTERNATIONAL FILING DATE 21 September 2000
	PRIORITY DATE CLAIMED 21 September 1999

TITLE OF INVENTION
PEPTIDES AGAINST AUTO-ANTIBODIES CAUSING DCM

APPLICANT(S) FOR DO/EO/US
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Applicant herein submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information.

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☒ This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).
4. ☒ A proper Demand for Internatl. Preliminary Examination was made by the 19th month from earliest claimed priority date.
5. ☒ A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   a. ☐ is transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☒ has been transmitted by the International Bureau.
   c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US)
6. ☒ A translation of the International Application into English (35 U.S.C. 371(c)(2)).
7. ☒ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
   a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☐ have been transmitted by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ☒ have not been made and will not be made.
8. ☐ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. ☐ An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
10. ☐ A translation of the annexes to the Internatl. Preliminary Examination report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11. to 16. below concern other document(s) or information included:

11. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. ☐ An assignment document for recording. A separate cover sheet compliance with 37 CFR 3.28 and 3.31 is included.
13. ☒ A FIRST preliminary amendment.
    ☐ A SECOND or SUBSEQUENT preliminary amendment.
14. ☐ A substitute specification.
15. ☐ A change of power of attorney and/or address letter.
16. ☒ Other items or information:
    International Search Report – EPO
    PCT/IB/301 Form
    PCT/IB/304 Form
    PCT/IB/308 Form
    First Page of Publication
    International Preliminary Examination Report – No Annexes

US APPLICATION NO (If known, see 37 CFR 1 5)	INTERNATIONAL APPLICATION NO	ATTORNEY'S DOCKET NUMBER
10/088681	PCT/EP00/09241	P67710US0

	CALCULATIONS	PTO USE ONLY
17. ☒ The following fees are submitted:		
Basic National Fee (37 CFR 1.492(a)(1)-(5)):		
Internatl. prelim. examination fee paid to USPTO (37 CFR 1.492 (a) (1)) . . $710.00		
No international preliminary examination fee paid to USPTO (37 CFR 1.492 (a) (2)) but international search fee paid to USPTO (37 CFR 1.445(a)(2)) . . $740.00		
Neither international preliminary examination fee (37 CFR 1.492 (a) (3)) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO) **$1040.00**		
International preliminary examination fee paid to USPTO (37 CFR 1.492 (a) (4)) and all claims satisfied provisions of PCT Article 33(2)-(4) $100.00		
Search Report prepared by the EPO or JPO (37 CFR 1.492 (a) (5)) **$890.00**		
ENTER APPROPRIATE BASIC FEE AMOUNT =	$ 890.00	
Surcharge of $130.00 for furnishing the **oath or declaration** later than ☐ **20** ☒ **30** months from the earliest claimed priority date (37 CFR 1.492(e)).	$ 130.00	

Claims	**Number Filed**	**Number Extra**	Rate		
Total Claims	**12** - 20 =	-0-	x $18.00	$	
Independent Claims	**1** - 3 =	-0-	x $84.00	$	
Multiple Dependent Claim(s) (if applicable)			+ $280.00	$	
TOTAL OF ABOVE CALCULATIONS =				$ 1020.00	
Reduction by 1/2 for filing by **small entity**, if applicable. Verified Small Entity statement must also be filed. (Note 37 CFR 1.9, 1.27, 1.28).				$	
SUBTOTAL =				$ 1020.00	
Processing fee of $130 for furnishing the **English translation** later than ☐ **20** ☐ **30** months from the earliest claimed priority date (37 CFR 1.492(f))				$	
TOTAL NATIONAL FEE =				$ 1020.00	
Fee of $40.00 for recording the enclosed **assignment** (37 CFR 1.21(h)). Assignment must be accompanied by appropriate cover sheet (37 CFR 3.28, 3.31).				$	
TOTAL FEES ENCLOSED =				$ 1020.00	
				Amt. to be refunded:	$
				Amt. charged:	$

a. ☒ A check in the amount of $ 1020.00 to cover the above fees is enclosed.

b. ☐ Please charge my Deposit Account No. **06-1358** in the amount of $ ____ to cover the above fees. A duplicate copy of this sheet is enclosed.

c. ☒ The Commissioner is hereby authorized to charge my account any additional fees set forth in §1.492 during the pendency of this application, or credit any overpayment to Deposit Account No. **06-1358**. A duplicate copy of this sheet is enclosed.

SEND ALL CORRESPONDENCE TO:

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By William E. Player
William E. Player
Reg. No. 31,409

JPH&S 3/95

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants: Wolfgang ROENSPECK et al

Serial No.: New

Filing Date: March 21, 2002

For: PEPTIDES AGAINST AUTO-ANTIBODIES CAUSING DCM

PRELIMINARY AMENDMENT

Assistant Commissioner of Patents
Washington, D.C. 20231

Sir:

Prior to initial examination, please amend the above-identified application as follows:

IN THE SPECIFICATION

Please insert the following sentence on line 1, immediately following the title:

--This is a 371 of PCT/EP00/09241, filed September 21, 2000, the disclosure of which is incorporated herein by reference.--

IN THE CLAIMS

Please cancel original claims 1-12 without prejudice or disclaimer.

Please add new claims 13-24 as found on the following pages.

CLAIMS:

13. A peptide having the amino acid sequence

X01-X02-X03-G-X04-X05-X06-X07-X08-X09-W-X10-X11-X12

wherein

X01 = amino group, acetyl group, biotin group, fluorescent label, spacer, linker or deletion;

X02 = D,G,E,T, S or deletion;

X03 = W,Y,F,G,T;

X04 = T,S,A,G;

X05 = L,F,Y,W;

X06 = V,I,W,F,Y;

X07 = S,A,C;

X08 = G,D,E,N,Q;

X09 = F,L,I,Y;

X10 = E,Q,T,S,L;

X11 = Y,F,T,S,W;

X12 = amide, the free acid, GKK, or a spacer;

and peptides having the amino acid sequence

X01-X02-W-X03-R-X04-X05-X06-X07-X08-E-A-R-X09-X10-X11-X12-X13-X14-X15-X16-X17

wherein

X01 = amino group, amino acid, peptide, acetyl group, biotin group,

[redacted]

fluorescent label, spacer, linker or deletion;

X02 = H, E, Q;

X03 = H, F, Y, W;

X04 = A, V;

X05 = G, T, E, S, D, N;

X06 = S, H, A;

X07 = D, N ,Q, E;

X08 = G, A, or a deletion;

X09 = D, N, R;

X10 = S, T, C, M;

X11 = H F, W, Y;

X12 = A, D, N, S;

X13 = D, N;

X14 = E, P;

X15 = R, K, T;

X16 = S, T, C, M or a deletion;

X17 = amide, the free acid, GKK, SGKK or a spacer.

14. The peptide according to claim 13 having the following amino acid sequence:

X01-X02-X03-G-X04-X05-X06-X07-X08-X09-W-X10-X11-X12

wherein

X01 = amino group, acetyl group, biotin group, fluorescent label, spacer, linker or deletion;

X02 = D,E,T, or deletion;

X03 = W,Y,T;

[redacted]

X04 = T,S;

X05 = L,F;

X06 = V,F;

X07 = S;

X08 = G,D,E;

X09 = F,L;

X10 = E,Q,T,L;

X11 = Y,T,S;

X12 = amid, the free acid, GKK, or a spacer;

and peptides having the amino acid sequence

X01-H-W-X03-R-A-X05-S-D-X08-E-A-R-R-S-Y-X12-D-P-X15-X16-X17

wherein

X01 = amino group, amino acid, peptide, acetyl group, biotin group, fluorescent label, spacer, linker or deletion;

X03 = Y, W;

X05 = T, E;

X08 = G, or a deletion;

X12 = A, N;

X15 = K, T;

X16 = S, or a deletion;

X17 = amide, the free acid, GKK, SGKK or a spacer.

15. The peptides according to claim 13 selected from the group consisting of:

-TGSFFSELWTSR[2],
EYGSFFSELWTSR[2],

[redacted]

TYGTLFSDFWLSR^2,
DWGTLVSGFWEYR^2,
DWGTLFSDFWQTR^2,

wherein R^2 is an acid amide, a free acid or GKKR^3, and wherein R^3 is an acid amide or a free acid;

with the proviso that a maximum of one non-conservative amino acid exchange is effected per amino acid position in the sequence, wherein "non-conservative exchange" means an exchange of amino acids between the groups mentioned below:

Group I: Leu, Ile, Val, Met, His, Trp, Tyr, Phe,

Group II: Glu, Gln, Asp, Asn,

Group III: Ser, Thr, Cys, Gly, Ala, Pro,

Group IV: Lys, Arg;

and

peptides selected from the group consisting of:

HWWRAESD-EARRSYNDPK-R^2,
HWYRATSDGEARRSYADPTSR^2,

with the proviso that a maximum of two non-conservative amino acid exchanges are effected per amino acid position in the sequence, wherein "non-conservative exchange" means an exchange of amino acids between the groups mentioned below:

Group I: Leu, Ile, Val, Met, His, Trp, Tyr, Phe,

Group II: Glu, Gln, Asp, Asn,

Group III: Ser, Thr, Cys, Gly, Ala, Pro,

Group IV: Lys, Arg.

TYGTLFSDFWLSR^2,
DWGTLVSGFWEYR^2,
DWGTLFSDFWQTR^2,

wherein R^2 is an acid amide, a free acid or GKKR^3, and wherein R^3 is an acid amide or a free acid;

with the proviso that a maximum of one non-conservative amino acid exchange is effected per amino acid position in the sequence, wherein "non-conservative exchange" means an exchange of amino acids between the groups mentioned below:

Group I: Leu, Ile, Val, Met, His, Trp, Tyr, Phe,

Group II: Glu, Gln, Asp, Asn;

Group III: Ser, Thr, Cys, Gly, Ala, Pro,

Group IV: Lys, Arg;

and

peptides selected from the group consisting of:

HWWRAESD-EARRSYNDPK-R^2,
HWYRATSDGEARRSYADPTSR^2,

with the proviso that a maximum of two non-conservative amino acid exchanges are effected per amino acid position in the sequence, wherein "non-conservative exchange" means an exchange of amino acids between the groups mentioned below:

Group I: Leu, Ile, Val, Met, His, Trp, Tyr, Phe,

Group II: Glu, Gln, Asp, Asn,

Group III: Ser, Thr, Cys, Gly, Ala, Pro,

Group IV: Lys, Arg.

16. The peptides according to claim 13, characterized by being:

TGSFF SELWT SGKK-amide or free acid,

E YGSFF SELWT SGKK-amide or free acid,

T YGTLF SDFWL SGKK-amide or free acid,

His-Trp-Trp-Arg-Ala-Glu-Ser-Asp-Glu-Ala-Arg-Arg-Ser-Tyr-Asn-Asp-Pro-Lys-amide or free acid,

Ala-Arg-Arg-Cys-Tyr-Asn-Asp-Pro-Lys-amide or free acid,

D WGTLV SGFWE Y amide or free acid,

D WGTLF SDFWQ TGKK amide or free acid,

H WYRAT SDGEA RRSYA DPTSG KK-amide or free acid,

HWWRAESDEARRSYNDPKC-amide or free acid,

which may also be acetylated N-terminally.

17. The peptides according to claim 13, characterized by being bound by antibodies of patients suffering from dilatative cardiomyopathy.

18. The peptides according to claim 13, characterized in that said linker is selected from the group consisting of:

- α-aminocarboxylic acids and their homo- and heterooligomers;
- α,ω-aminocarboxylic acids and their branched homo- or heterooligomers;
- other amino acids and their linear and branched homo- or heterooligomers (peptides);
- amino-oligoalkoxy-alkylamines;
- maleinimidocarboxylic acid derivatives;
- oligomers of alkylamines;
- 4-alkylphenyl derivatives;
- 4-oligoalkoxyphenyl or 4–oligoalkoxyphenoxy derivatives;
- 4-oligoalkylmercaptophenyl or 4–oligoalkylmercaptophenoxy derivatives;

- 4-oligoalkylaminophenyl or 4-oligoalkylaminophenoxy derivatives;
- (oligoalkylbenzyl)phenyl or (4-oligoalkylbenzyl)phenoxy derivatives, and (4-oligoalkoxybenzyl)phenyl or (4-oligoalkoxybenzyl)phenoxy derivatives;
- trityl derivatives;
- benzyloxyaryl or benzyloxyalkyl derivatives;
- xanthene-3-yloxyalkyl derivatives;
- (4-alkylphenyl) or ω-(4-alkylphenoxy)alkanoic acid derivatives;
- oligoalkylphenoxyalkyl or oligoalkoxyphenoxyalkyl derivatives;
- carbamate derivatives;
- amines;
- trialkylsilyl or dialkylalkoxysilyl derivatives;
- alkyl or aryl derivatives;
- and combinations thereof.

19. The peptides according to claim 13, characterized by being bound to a solid phase.

20. The peptides according to claim 13, characterized by being bound to a solid phase through a spacer.

21. A medicament containing the peptides according to claim 13.

22. Use of the peptides according to claim 13 for the preparation of a medicament for treatment with diseases related to β_1-adrenergically active auto-antibodies, especially dilatative cardiomyopathy.

23. A method for treating diseases related to β_1-adrenergically active auto-antibodies by removing the auto-antibodies by means of peptides according to claim 18 bound to a solid phase.

24. A device for chromatography containing peptides according to claim 18 bound to a solid phase.

REMARKS

The foregoing Preliminary Amendment is requested in order to delete the multiple dependent claims and avoid paying the multiple dependent claims fee.

Early action on the merits is respectfully requested.

Respectfully submitted,

JACOBSON HOLMAN PLLC

By _____
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Atty. Docket: P67710US0
Date: March 21, 2002
WEP:jrc

SMB

Peptides Against Auto-Antibodies Causing DCM

The present invention relates to peptides against auto-antibodies causing DCM, medicaments containing such peptides, the use of the peptides, methods for the treatment of diseases related to β_1-adrenergically active auto-antibodies, and a device for immunoadsorption containing the peptides bound to a solid phase.

The immune system is an essential component of all animal beings. In mammals, in particular, it serves for defense against microorganisms, tissue regeneration and destruction of tumor cells. In classical immunology, distinction is made between cellular and humoral immune defense. This means two distinguishable, but cooperating systems which ultimately represent the immune system.

A number of diseases exist which, due to their pathogenesis, are considered auto-immune diseases. In such diseases, the immune system of the afflicted subjects is directed against their own organs, tissues, cells or proteins and other molecules. The predominantly cell-mediated auto-immune diseases include multiple sclerosis and diabetes (type I).

A second group are the predominantly antibody-mediated auto-immune diseases. These include, for example, rheumatism, the less frequently occurring auto-immune diseases, such as myasthenia gravis or lupus erythematodes, and recently also dilatative cardiomyopathy (DCM).

The pathogenesis of most auto-immune diseases is unknown. There are various hypotheses and models of how to explain the genesis of auto-immune diseases. One explaining model is antigenic/molecular mimicry. In this model, it is

considered that microorganisms, e.g., viruses or parasites, provide themselves with particular molecules which are, for example, remarkably similar to or even in part identical with endogenous structures of the host and are therefore not recognized by the immune system of the host.

However, when they are recognized as foreign and antibodies are produced against them, then such antibodies will also recognize similar endogenous structures, which results in activation of the immune system and complement system. This induces pathological reactions in situ in the tissue, for example, chronic inflammations, or a pathological dysfunction of the cells occurs to which the auto-antibodies have bound.

Dilatative cardiomyopathy can be considered a prominent example thereof. In this auto-immune disease, the organisms erroneously forms antibodies which bind to defined regions of β_1-adrenergic receptor. These regions are on the first and second loops of a total of three extracellular loops of β_1-adrenergic receptor.

Such auto-antibodies which are capable to bind to these regions cause an increase of the pulsation rate in biological tests with rat cardiomyocytes in a cell culture (these cells have a nearly identical β_1-adrenergic receptor on their surface). This is referred to as a pharmaco-active effect of the auto-antibodies similar to that of adrenalin. The auto-antibodies directed against the epitopes on loops 1 and 2 of the β_1-adrenergic receptor are mainly observed in patients suffering from DCM. Occasionally, such auto-antibodies are also observed in patients having cardiac dysrhythmia and myocarditis.

Dilatative cardiomyopathy is an auto-immune disease which, when not treated, results in a severe deterioration of the cardiac output, i.e., reduction of the pumping output with simultaneous expansion of the myocardiac tissue by infiltrates, and then in heart transplantation or death.

However, if the antibodies are removed from the patient's blood by lavage of the blood, regeneration of the heart muscle and a dramatic improvement of the

myocardiac output, which almost reaches the values of healthy people, occur within one year.

In patients with DCM, an immunoglobulin fraction which contains the specific auto-antibodies binding to β_1-adrenergic receptor and thereby activate the cell can be isolated from the plasma. When peptides of β_1-adrenergic receptor which represent the binding site for the auto-antibodies are added to a cell culture of rat cardiomyocytes, the pathological effect of the immunoglobulin fraction can be neutralized.

If the same peptides which correspond to the native sequences are coupled to a solid phase, they are no longer capable of binding and eliminating the auto-antibodies described from a patient's blood plasma. This means that the peptides which correspond to the native sequence of β_1-adrenergic receptor and represent binding sites for the pathological auto-antibodies described cannot be used for immuno-adsorption.

It has been the object of the invention to provide peptides which recognize, bind and eliminate pathological auto-antibodies directed against functional epitopes in the blood or plasma of patients having a positive antibody state or DCM, wherein the peptides, in addition to the epitopes which respectively neutralize the antibody effect, simultaneously contain amino acid sequences which enable binding of the pathological antibodies.

Surprisingly, this object is achieved by peptides having the amino acid sequence

X01-X02-X03-G-X04-X05-X06-X07-X08-X09-W-X10-X11-X12

wherein

X01 = amino group, acetyl group, biotin group, fluorescent label, spacer, linker or deletion;

X02 = D,G,E,T, S or deletion;

X03 = W,Y,F,G,T;

X04 = T,S,A,G;

X05 = L,F,Y,W;

X06 = V,I,W,F,Y;

X07 = S,A,C;

X08 = G,D,E,N,Q;

X09 = F,L,I,Y;

X10 = E,Q,T,S,L;

X11 = Y,F,T,S,W;

X12 = amide, the free acid, GKK, or a spacer;

and peptides having the amino acid sequence

X01-X02-W-X03-R-X04-X05-X06-X07-X08-E-A-R-X09-X10-X11-X12-X13-X14-X15-X16-X17

wherein

X01 = amino group, amino acid, peptide, acetyl group, biotin group, fluorescent label, spacer, linker or deletion;

X02 = H, E, Q;

X03 = H, F, Y, W;

X04 = A, V;

X05 = G, T, E, S, D, N;

X06 = S, H, A;

X07 = D, N ,Q, E;

X08 = G, A, or a deletion;

X09 = D, N, R;

X10 = S, T, C, M;

X11 = H F, W, Y;

X12 = A, D, N, S;

X13 = D, N;

X14 = E, P;

X15 = R, K, T;

X16 = S, T, C, M or a deletion;

X17 = amide, the free acid, GKK, SGKK or a spacer.

In particular, peptides according to the invention having the following amino acid sequence are employed:

X01-X02-X03-G-X04-X05-X06-X07-X08-X09-W-X10-X11-X12

wherein

X01 = amino group, acetyl group, biotin group, fluorescent label, spacer, linker or deletion;

X02 = D,E,T, or deletion;

X03 = W,Y,T;

X04 = T,S;

X05 = L,F;

X06 = V,F;

X07 = S;

X08 = G,D,E;

X09 = F,L;

X10 = E,Q,T,L;

X11 = Y,T,S;

X12 = amid, the free acid, GKK, or a spacer;

and peptides having the amino acid sequence

X01-H-W-X03-R-A-X05-S-D-X08-E-A-R-R-S-Y-X12-D-P-X15-X16-X17

wherein

X01 = amino group, amino acid, peptide, acetyl group, biotin group, fluorescent label, spacer, linker or deletion;

X03 = Y, W;

X05 = T, E;

X08 = G, or a deletion;

X12 = A, N;

X15 = K, T;

X16 = S, or a deletion;

X17 = amide, the free acid, GKK, SGKK or a spacer.

It is particularly preferred to employ the following peptides which are selected from the group consisting of

-TGSFFSELWTSR^2,
EYGSFFSELWTSR^2,
TYGTLFSDFWLSR^2,
DWGTLVSGFWEYR^2,
DWGTLFSDFWQTR^2,

wherein R^2 is an acid amide, a free acid or GKKR^3, and wherein R^3 is an acid amide or a free acid;

with the proviso that a maximum of one non-conservative amino acid exchange is effected per amino acid position in the sequence, wherein "non-conservative exchange" means an exchange of amino acids between the groups mentioned below:

Group I: Leu, Ile, Val, Met, His, Trp, Tyr, Phe,

Group II: Glu, Gln, Asp, Asn,

Group III: Ser, Thr, Cys, Gly, Ala, Pro,

Group IV: Lys, Arg;

and

peptides selected from the group consisting of:

HWWRAESD-EARRSYNDPK-R^2,
HWYRATSDGEARRSYADPTSR^2,

with the proviso that a maximum of two non-conservative amino acid exchanges are effected per amino acid position in the sequence, wherein "non-conservative exchange" means an exchange of amino acids between the groups mentioned below:

Group I: Leu, Ile, Val, Met, His, Trp, Tyr, Phe,

Group II: Glu, Gln, Asp, Asn,

Group III: Ser, Thr, Cys, Gly, Ala, Pro,

Group IV: Lys, Arg.

The following peptides are particularly preferred:

TGSFF SELWT SGKK-amide or free acid, (SEQ. ID. NO. 1)

E YGSFF SELWT SGKK-amide or free acid, (SEQ. ID. NO. 2)

T YGTLF SDFWL SGKK-amide or free acid, (SEQ. ID. NO. 3)

His-Trp-Trp-Arg-Ala-Glu-Ser-Asp-Glu-Ala-Arg-Arg-Ser-Tyr-Asn-Asp-Pro-Lys-amide or free acid, (SEQ. ID. NO. 4)

Ala-Arg-Arg-Cys-Tyr-Asn-Asp-Pro-Lys-amide or free acid, (SEQ. ID. NO. 5)

D WGTLV SGFWE Y amide or free acid, (SEQ. ID. NO. 6)

D WGTLF SDFWQ TGKK amide or free acid, (SEQ. ID. NO. 7)

H WYRAT SDGEA RRSYA DPTSG KK-amide or free acid, (SEQ. ID. NO. 8)

HWWRAESDEARRSYNDPKC-amide or free acid, (SEQ. ID. NO. 9)

which may also be acetylated N-terminally.

The skilled person knows that amino acid positions in peptides or proteins can be exchanged in a conservative manner without affecting the function. In the

present case, a "conservative" exchange means an exchange within the groups set forth below:

Group I: Leu, Ile, Val, Met, His, Trp, Tyr, Phe,

Group II: Glu, Gln, Asp, Asn,

Group III: Ser, Thr, Cys, Gly, Ala, Pro,

Group IV: Lys, Arg.

The peptides according to the invention are bound, in particular, by antibodies of patients suffering from dilatative cardiomyopathy.

As a linker according to the invention, all structures may be used which are available for that purpose unless adversely affecting the binding behavior of the peptides towards the antibodies. Usually, a linker is a chemical compound which provides at least one linking site (functional group) on a polymeric matrix which is otherwise free of functions.

The linking site serves for coupling a ligand or a spacer and matches the chemical properties of the ligand or spacer. Such a link is stable or cleavable depending on the type of linker molecule.

A ligand is usually a compound having some special property. According to the invention, the ligand is preferably a peptide which is capable of specifically binding an auto-antibody which has an adrenergic activity and is directed against the β_1-adrenergic receptor of the heart muscle.

According to the invention, the following linkers are preferably employed:

α-aminocarboxylic acids and their homo- and heterooligomers, α,ω-amino-carboxylic acids and their branched homo- or heterooligomers, other amino acids and their linear and branched homo- or heterooligomers (peptides); amino-oligoalkoxy-alkylamines; maleinimidocarboxylic acid derivatives; oligomers of alkylamines; 4-alkylphenyl derivatives; 4-oligoalkoxyphenyl or 4–oligoalkoxy-

phenoxy derivatives; 4-oligoalkylmercaptophenyl or 4–oligoalkylmercaptophenoxy derivatives; 4-oligoalkylaminophenyl or 4–oligoalkylaminophenoxy derivatives; (oligoalkylbenzyl)phenyl or (4–oligoalkylbenzyl)phenoxy derivatives, and (4–oligoalkoxybenzyl)phenyl or (4–oligoalkoxybenzyl)phenoxy derivatives; trityl derivatives; benzyloxyaryl or benzyloxyalkyl derivatives; xanthene-3-yloxyalkyl derivatives; (4-alkylphenyl) or ω-(4-alkylphenoxy)alkanoic acid derivatives; oligoalkylphenoxyalkyl or oligoalkoxyphenoxyalkyl derivatives; carbamate derivatives; amines; trialkylsilyl or dialkylalkoxysilyl derivatives; alkyl or aryl derivatives, and combinations thereof.

In particular, the peptides according to the invention are bound to a solid phase for use. Preferably, the binding of the peptides to the solid phase is effected through a spacer. As the spacer, there may be used virtually all chemical compounds or groups suitable for such a function unless adversely affecting the binding behavior to such an extent that binding of the antibody with the peptide is prevented or substantially impaired.

A spacer is usually a compound which is inserted between a ligand and a linker if necessary and serves for positioning the ligand at a distance and in a spatial position appropriate for the binding of the auto-antibody. Spacers are molecules having at least two chemically active groups (functional groups), of which one group binds to the linker molecule, and at least one second functional group mediates binding to a ligand. By selecting the spacer, an increase of flexibility and an improvement of accessibility as well as an oriented arrangement of the ligands and increase of ligand density on the surface can be achieved depending on requirements.

Spacers include, for example, ω-aminocarboxylic acids and their homo- and heterooligomers, α,ω-aminocarboxylic acids and their branched homo- or heterooligomers, other aminocarboxylic acids and their linear and branched homo- or heterooligomers, maleinimidocarboxylic acid derivatives, hydroxycarboxylic acid derivatives, dicarboxylic acid derivatives, diamine derivatives dihydroxyalkyl derivatives, and hydroxyalkylamine derivatives. Preferably, mono- or dioligomers of β-alanine or ω-aminohexanoic acid and branched mono- or dioligomers

of lysine or ornithine are used. The technology by means of which peptides can be anchored to solid phases is per se known to the skilled person.

In another embodiment of the invention, the peptides according to the invention are employed as medicaments.

In this concept, peptides are particularly altered (e.g. by cyclization) so that they cannot be destroyed by serum proteases and will bind antibodies in solutions. In this way, in-vivo neutralization of the antibodies can take place by intravenously administering the correspondingly processed peptides. The peptides are to be considered as medicaments herein. Their development is directly derived from the peptides binding the antibodies, which are fixed to a column matrix.

The amount of peptides to be administered depends on their molecular weight (i.e., their size) and on the concentration of auto-antibodies which can be reached in the blood stream and other compartments. According to what is known today, the amounts of auto-antibodies are within the µg and ng ranges. A quantity of between 1 and 5 µg of peptide should be sufficient for binding the antibodies present and pass them to elimination as an immune complex in accordance with the natural clearance mechanisms. Subsequently, the dosages can be lower since only the newly produced antibodies must be eliminated.

In principle, other dosage forms may be suitable in this case too. When the corresponding galenic methods are used, absorption in the intestine of peptides binding β_1-adrenergic antibodies should be achievable. In this case, the dosages would preferably be higher by a factor of about 10 to 20.

The peptides according to the invention can be employed for the preparation of a medicament for treatment of diseases related to β_1-adrenergically active auto-antibodies, especially dilatative cardiomyopathy, blood hypertension diseases, and a form of cardiomyopathy induced by *Trypanosoma cruzi*. In addition to dilatative cardiomyopathy, a number of diseases exist which can be classified as auto-immune diseases and are subject to similar pathomechanisms. Also for pre-eclampsia and certain forms of malignant blood hypertension, auto-antibodies

have been described which contribute to the hyperactivation of cells by stimulating their angiotensin receptor or α_1 receptor, and participate in the genesis of defined clinical pictures. The use of peptides for eliminating these specific auto-antibodies or for in-vivo neutralization of the auto-antibodies can be regarded by analogy with dilatative cardiomyopathy.

According to the invention, a method is claimed for the treatment of diseases related to β_1-adrenergically active auto-antibodies by removing the auto-antibodies using peptides bound to a solid phase. Advantageously, this may be effected with a device for chromatography according to the invention containing the peptides according to the invention bound to a solid phase.

The peptides are fixed to a solid phase, e.g., sepharose, in a closed sterile container which usually has a volume of between 5 and 250 ml. In this sterile space, the patients' blood plasma, from which the cells have previously been removed by a medical engineering apparatus, flows over or through the adsorption matrix, i.e., the peptide-coated sepharose surface. This results in the binding of the pathological auto-antibodies to the peptides which simulate regions of the β_1-adrenergic receptor. If suitable adsorption matrices are employed, the previous separation of the cells can be dispensed with.

The remaining components of the blood plasma or blood including all necessary and useful immunoglobulins then leave the column and are recirculated into the patient's blood stream. This device for extracorporeal therapy is state of the art as far as the non-specific elimination of plasma proteins or immunoglobulin is concerned.

The device according to the invention uses the peptides derived from the β_1-adrenergic receptor for removing the small but pathologically relevant auto-antibody fraction from the plasma.

After a certain amount of blood plasma has flown through the device, the blood plasma stream can be switched over to a second column which is technically identical with the first, while the first column is being regenerated. That means,

the loaded antibodies are separated from the peptide and discarded by using different rinsing and elution solutions, preferably physiological saline with and without additional buffering, for example, by phosphate, glycine or citrate, and a pH range of from pH 2 to pH 7.5. Subsequently, the thus regenerated column is again available for the binding and elimination of the pathological auto-antibodies from the patient's blood plasma. This double column principle has proven useful and is always used when regeneration of the column is required.

A second variant of the use of peptides for the elimination of pathological auto-antibodies includes the use of disposable columns. In these columns, the solid-phase matrix contains an amount of peptide sufficient that major portions of the auto-antibodies can be removed from the plasma in several hours of treatment. The advantage resides in the fact that the time-consuming and tedious regeneration of the adsorption matrix can be dispensed with.

A third variant of the treatment of DCM patients by the elimination of pathological antibodies from the blood plasma includes the use of columns in which a previous separation of plasma and blood cells is not required due to the design of the columns.

The use of columns requires technical devices which ensure a blood and plasma input and flow adequate for the treatment by using different flexible tubes, pumps, monitor screens and other monitoring systems.

Example

1. Peptides

The following two peptides were immobilized on cross-linked agarose beads Sepharose 4B as the solid phase.

Peptide 1: TGSFFCELWTSGKK
Peptide 2: HWWRAESDEARRSYNDPKC

Sepharose CL4B served as the filling matrix. From the two peptide matrices and the filling matrix, an affinity chromatographic column was prepared, and its function, i.e., the removal of DCM-related auto-antibodies from human plasma, was tested with human plasma as a sample.

2. Immobilization of the peptides

For immobilization on a solid phase, the peptides were dissolved in coupling buffer (0.5 M NaCl, 0.1 M NaHCO3, pH 8.3) to a concentration of 2 mg/ml, and mixed with washed and CNBr-preactivated Sepharose 4B. After completion of the coupling reaction, the peptide matrices were washed with coupling buffer, and the excess CNBr groups were inactivated.

The peptide loading of the matrices was determined photometrically by calculating the difference between the mass of peptide employed before the coupling and the mass of non-immobilized peptide after the coupling.

The matrix loading with peptide 1 was 2.0 mg of peptide/ml of matrix. The matrix loading with peptide 2 was 1.8 mg of peptide/ml of matrix.

To prepare the affinity chromatographic column, peptide matrix 1 and peptide matrix 2 were mixed at a ratio of 1:1, and this mixture was mixed with filling matrix at a ratio of 1:5. The total volume of the matrix for the affinity chromatographic column was 100 ml.

3. Removal of DCM-related auto-antibodies from human plasma

The function of the above described affinity chromatographic column was tested on two persons afflicted with DCM and exhibiting a positive test for DCM-related auto-antibodies.

Prior to the application, the persons must be treated with anti-coagulants, such as heparin, hirudin or citrate.

Thus, from the recommended concentration range of from 1500 to 3000 units, a bolus of heparin of 2000 units was chosen for intravenous administration. During the application, from the recommended concentration range of from 250 to 750 units of heparin per hour, a dosage of 500 units of heparin per hour was chosen for intravenous administration.

The blood was passed into a separator in which the separation of the cellular components of the blood from the blood plasma was effected. The plasma of the persons testing positive for DCM-related auto-antibodies was passed over the affinity chromatographic column. The volume ratio of the affinity matrix to the plasma was between 1:6 and 1:10 for the affinity chromatographic column runs performed. All in all, 20 such purification cycles were performed, wherein the treated plasma was reinfused to the persons after each cycle together with the cellular blood components. The total number of purification cycles of 20 is derived from 4 purification cycles per day of application for a total of 5 days of application.

4. Quantitative assay of the DCM-related auto-antibodies

A quantitative assay of the DCM-related auto-antibodies was effected from plasma samples of the persons afflicted with DCM, obtained before and after the application on each day of application. The assay of the DCM-related auto-antibodies (antibodies against β_1-adrenergic receptor) was performed according to Wallukat, G., Wollenberger, A., Morwinski, R. and Pitschner, H.F. (1995); Anti-β_1-adrenoceptor antibodies with chronotropic activity from the serum of patients with dilated cardiomyopathy: mapping of epitopes in the first and second extracellular loops; J. Mol. Cell. Cardiol. 27, 397-406.

5. Results of the removal of DCM-related Auto-Antibodies from Human Plasma

For person 1 having auto-antibodies which preferably bind to peptide 1, a reduction of auto-antibodies to 12% of the initial value was achieved after completion of the overall application.

For person 2 having auto-antibodies which preferably bind to peptide 2, a reduction of auto-antibodies to 5% of the initial value was achieved after completion of the overall application.

The results for the overall application are set forth in Table 1.

In both persons, the concentrations of other plasma parameters, such as total protein, albumin and IgG, were almost unaffected during the overall application.

Table 1: DCM-related auto-antibodies (antibodies against β_1-adrenergic receptor) in the human plasma of person 1 and person 2 before, during and after treatment with the affinity chromatographic column.

After elution from the affinity matrix, the auto-antibodies bound to the affinity chromatographic column were tested for preferential binding to peptide 1 or peptide 2 by means of peptide 1 and peptide 2 bound to a solid phase. Auto-antibody type 1 prefers the binding of peptide 1, and auto-antibody type 2 prefers the binding of peptide 2.

Person 1

Plasma sample	Auto-antibody 2 [relative units]	Auto-antibody 2 [%]
before cycle 1-4	5.8	100
after cycle 1-4	2.4	41
before cycle 5-8	3.4	59
after cycle 5-8	1.6	28
before cycle 9-12	3.3	57
after cycle 9-12	2.0	34
before cycle 13-16	1.9	33
after cycle 13-16	0.8	14
before cycle 17-20	1.7	29
after cycle 17-20	0.7	12

Person 2

Plasma sample	Auto-antibody 2 [relative units]	Auto-antibody 2 [%]
before cycle 1-4	6.1	100
after cycle 1-4	3.1	51
before cycle 5-8	3.8	62
after cycle 5-8	3.0	49
before cycle 9-12	3.5	57
after cycle 9-12	2.3	38
before cycle 13-16	2.8	46
after cycle 13-16	0.9	15
before cycle 17-20	1.7	28
after cycle 17-20	0.3	5

CLAIMS:

1. A peptide having the amino acid sequence

X01-X02-X03-G-X04-X05-X06-X07-X08-X09-W-X10-X11-X12

wherein

X01 = amino group, acetyl group, biotin group, fluorescent label, spacer, linker or deletion;

X02 = D,G,E,T, S or deletion;

X03 = W,Y,F,G,T;

X04 = T,S,A,G;

X05 = L,F,Y,W;

X06 = V,I,W,F,Y;

X07 = S,A,C;

X08 = G,D,E,N,Q;

X09 = F,L,I,Y;

X10 = E,Q,T,S,L;

X11 = Y,F,T,S,W;

X12 = amide, the free acid, GKK, or a spacer;

and peptides having the amino acid sequence

X01-X02-W-X03-R-X04-X05-X06-X07-X08-E-A-R-X09-X10-X11-X12-X13-X14-X15-X16-X17

wherein

X01 = amino group, amino acid, peptide, acetyl group, biotin group, fluorescent label, spacer, linker or deletion;

X02 = H, E, Q;

X03 = H, F, Y, W;

X04 = A, V;

X05 = G, T, E, S, D, N;

X06 = S, H, A;

X07 = D, N ,Q, E;

X08 = G, A, or a deletion;

X09 = D, N, R;

X10 = S, T, C, M;

X11 = H F, W, Y;

X12 = A, D, N, S;

X13 = D, N;

X14 = E, P;

X15 = R, K, T;

X16 = S, T, C, M or a deletion;

X17 = amide, the free acid, GKK, SGKK or a spacer.

2. The peptide according to claim 1 having the following amino acid sequence:

X01-X02-X03-G-X04-X05-X06-X07-X08-X09-W-X10-X11-X12

wherein

X01 = amino group, acetyl group, biotin group, fluorescent label, spacer, linker or deletion;

X02 = D,E,T, or deletion;

X03 = W,Y,T;

X04 = T,S;

X05 = L,F;

X06 = V,F;

X07 = S;

X08 = G,D,E;

X09 = F,L;

X10 = E,Q,T,L;

X11 = Y,T,S;

X12 = amid, the free acid, GKK, or a spacer;

and peptides having the amino acid sequence

X01-H-W-X03-R-A-X05-S-D-X08-E-A-R-R-S-Y-X12-D-P-X15-X16-X17

wherein

X01 = amino group, amino acid, peptide, acetyl group, biotin group, fluorescent label, spacer, linker or deletion;

X03 = Y, W;

X05 = T, E;

X08 = G, or a deletion;

X12 = A, N;

X15 = K, T;

X16 = S, or a deletion;

X17 = amide, the free acid, GKK, SGKK or a spacer.

3. The peptides according to any of claims 1 or 2 selected from the group consisting of:

-TGSFFSELWTSR^2,
EYGSFFSELWTSR^2,
TYGTLFSDFWLSR^2,

DWGTLVSGFWEYR^2,
DWGTLFSDFWQTR^2,

wherein R^2 is an acid amide, a free acid or GKKR^3, and wherein R^3 is an acid amide or a free acid;

with the proviso that a maximum of one non-conservative amino acid exchange is effected per amino acid position in the sequence, wherein "non-conservative exchange" means an exchange of amino acids between the groups mentioned below:

Group I: Leu, Ile, Val, Met, His, Trp, Tyr, Phe,

Group II: Glu, Gln, Asp, Asn,

Group III: Ser, Thr, Cys, Gly, Ala, Pro,

Group IV: Lys, Arg;

and

peptides selected from the group consisting of:

HWWRAESD-EARRSYNDPK-R^2,
HWYRATSDGEARRSYADPTSR^2,

with the proviso that a maximum of two non-conservative amino acid exchanges are effected per amino acid position in the sequence, wherein "non-conservative exchange" means an exchange of amino acids between the groups mentioned below:

Group I: Leu, Ile, Val, Met, His, Trp, Tyr, Phe,

Group II: Glu, Gln, Asp, Asn,

Group III: Ser, Thr, Cys, Gly, Ala, Pro,

Group IV: Lys, Arg.

4. The peptides according to claims 1 to 3, characterized by being:

TGSFF SELWT SGKK-amide or free acid,

E YGSFF SELWT SGKK-amide or free acid,

T YGTLF SDFWL SGKK-amide or free acid,

His-Trp-Trp-Arg-Ala-Glu-Ser-Asp-Glu-Ala-Arg-Arg-Ser-Tyr-Asn-Asp-Pro-Lys-amide or free acid,

Ala-Arg-Arg-Cys-Tyr-Asn-Asp-Pro-Lys-amide or free acid,

D WGTLV SGFWE Y amide or free acid,

D WGTLF SDFWQ TGKK amide or free acid,

H WYRAT SDGEA RRSYA DPTSG KK-amide or free acid,

HWWRAESDEARRSYNDPKC-amide or free acid,

which may also be acetylated N-terminally.

5. The peptides according to claims 1 to 4, characterized by being bound by antibodies of patients suffering from dilatative cardiomyopathy.

6. The peptides according to any of claims 1 to 5, characterized in that said linker is selected from the group consisting of:

- α-aminocarboxylic acids and their homo- and heterooligomers;
- α,ω-aminocarboxylic acids and their branched homo- or heterooligomers;
- other amino acids and their linear and branched homo- or heterooligomers (peptides);
- amino-oligoalkoxy-alkylamines;
- maleinimidocarboxylic acid derivatives;
- oligomers of alkylamines;
- 4-alkylphenyl derivatives;
- 4-oligoalkoxyphenyl or 4–oligoalkoxyphenoxy derivatives;
- 4-oligoalkylmercaptophenyl or 4–oligoalkylmercaptophenoxy derivatives;

- 4-oligoalkylaminophenyl or 4-oligoalkylaminophenoxy derivatives;
- (oligoalkylbenzyl)phenyl or (4-oligoalkylbenzyl)phenoxy derivatives, and (4-oligoalkoxybenzyl)phenyl or (4-oligoalkoxybenzyl)phenoxy derivatives;
- trityl derivatives;
- benzyloxyaryl or benzyloxyalkyl derivatives;
- xanthene-3-yloxyalkyl derivatives;
- (4-alkylphenyl) or ω-(4-alkylphenoxy)alkanoic acid derivatives;
- oligoalkylphenoxyalkyl or oligoalkoxyphenoxyalkyl derivatives;
- carbamate derivatives;
- amines;
- trialkylsilyl or dialkylalkoxysilyl derivatives;
- alkyl or aryl derivatives;
- and combinations thereof.

7. The peptides according to any of claims 1 to 6, characterized by being bound to a solid phase.

8. The peptides according to any of claims 1 to 7, characterized by being bound to a solid phase through a spacer.

9. A medicament containing the peptides according to any of claims 1 to 8.

10. Use of the peptides according to any of claims 1 to 8 for the preparation of a medicament for treatment with diseases related to β_1-adrenergically active auto-antibodies, especially dilatative cardiomyopathy.

11. A method for treating diseases related to β_1-adrenergically active auto-antibodies by removing the auto-antibodies by means of peptides according to claim 6 or 7 bound to a solid phase.

12. A device for chromatography containing peptides according to claim 6 or 7 bound to a solid phase.

Abstract

Peptides which bind auto-antibodies being in a causally pathological relationship with dilatative cardiomyopathy are described. The peptides may be bound, for example, to a solid phase. Auto-antibodies can be removed by treating blood of patients suffering from DCM with the peptides according to the invention.

DECLARATION AND POWER OF ATTORNEY U.S.A.

ALL PATENTS, INCLUDING DESIGN
FOR APPLICATION BASED ON PCT; PARIS CONVENTION;
NON PRIORITY; OR PROVISIONAL APPLICATIONS

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101 102

As a below named inventor, I declare that my residence, post office address and citizenship are stated below next to my name, the information given herein is true, that I believe that I am the original, first and sole inventor (if only one name is listed at 201 below), or an original, first and joint inventor (if plural inventors are named below at 201-203, or on additional sheets attached hereto) of the subject matter which is claimed and for which patent is sought on the invention entitled:

Peptides Against Auto-Antibodies Causing DCM

which is described and claimed in:

- [X] PCT International Application No. PCT/EP 00/09241 filed Sept. 21, 2000
- [ ] the attached specification
- [ ] the specification in application Serial No. _____ filed _____

(if applicable) and amended on _____

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.
I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, §1.56.
I hereby claim foreign priority benefits under Title 35, United States Code, §119 (a)-(d) of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed:

103 104

Prior Foreign Application(s) (Number)	(Country)	(Day/Month/Year Filed)	Priority Claimed
99 118 630.5	EP	Sept. 21, 1999	[X] Yes [ ] No
99 118 631.3	EP	Sept. 21, 1999	[X] Yes [ ] No
			[ ] Yes [ ] No

I hereby claim the benefit under Title 35, United States Code, §119(e) of any United States provisional application(s) listed below:

Application No. _____ Filing Date _____ Application No. _____ Filing Date _____

105

I hereby claim the benefit under Title 35, United States Code, §120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, §1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application:

(Application Serial No.)	(Filing Date)	(Status: patented, pending, abandoned)

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I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are to be true, and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment or both, under section 1001 of Title 18 of the United States Code; and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

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